

A gutless wonder: first record of the remarkable acoel flatworm *Wulguru cuspidata* in the Northern Territory

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Abstract

This paper records the microscopic acoel flatworm *Wulguru cuspidata* from the Northern Territory, in fact from the northern coastline of Australia, for the first time. Whilst being restricted to a narrow belt in the intertidal zone on sandy beaches fronting the ocean, this acoel can attain remarkably high densities. Despite the stressors it must experience in this habitat – of great fluctuations of temperature and salinity, and of toxic algal and bacterial blooms – it is present year round. Its behaviour, which is typical of acoels of the family Convolutidae, of moving to the surface of the substrate at low tide apparently to recharge its ‘solar batteries’ (i.e. provide light for its symbiotic microalgae) and of burying into the substrate at high tide, is described. Whilst this behaviour appears to be a response to a combination of tidal and daylight cycles, the biological clock causing it must be very complicated.

Introduction

In early July 2014, one of us (Megan Hoskins) observed black ‘smudges’ in wave-formed ripples and water-formed runnels on the intertidal sand flats in the vicinity of the mouth of Buffalo Creek, in southern Shoal Bay, on the south-eastern coast of Beagle Gulf, Northern Territory. The ‘smudges’, that were present only in one narrow belt on the tidal flats, looked as though they had been formed by coffee grounds or coal dust sprinkled on the clean quartz sand. At other times of the year the ‘smudges’ appeared as intense 10 mm wide black spots on the floor of these puddles (Figures 1, 2, 7–9). On close inspection, the ‘smudges’ were revealed as consisting of hundreds of thousands of actively moving, microscopic, dark green ‘worms’ (Figures 3, 4). It is remarkable that they were never noticed before.

The 'worms' were recognised as acoels of the family Convolutidae when they were examined under a microscope by Richard Willan. Field trips made by us during July indicated these acoels were present, and in equally high densities, throughout Shoal Bay and the outer (more oceanic) section of Darwin Harbour. The observations reported in this paper were made both at the mouth of Buffalo Creek (12.33°S, 130.90°E) and at several sites at Casuarina Beach (12.35°S, 130.87°E), particularly at the mouths of Rapid Creek, Surf Lifesaver Creek and Sandy Creek (Figure 1).

All four authors continued to observe the acoels throughout the dry season, the 'build-up' and the early wet season of 2014, up to the first bout of the north-westerly monsoon in January 2015. We expected the populations would vanish sometime during this period when the air or water temperature increased, or when the annual bloom of *Trichodesmium* cyanobacteria choked the intertidal zone, or when the puddles became filled with the annual die-off of *Sargassum* sp. seaweed, or when the salinity fell to almost zero after bursts of heavy rain, or when the monsoon set in. But they persisted throughout all these environmental 'catastrophes'.



Figure 1. *Wulguru cuspidata* inhabits water-filled wave-formed runnels on open sandy beaches north of Darwin. This photograph shows the habitat near the mouth of Sandy Creek at Casuarina Beach with the transition between the runnels on the innermost mid-tidal sand flats (to the left of centre) to the smooth sloping high-tidal beach (to the right of centre). The direction of view is due north, 24 October 2014. (Neil Wright)





Figure 2. Aggregations of *Wulguru cuspidata* appear as dark sprinklings in wave-formed sand ripples on the clean quartz sand at mid-tidal level. Buffalo Creek, Shoal Bay, 23 December 2014. (Megan Hoskins) ◀

Figure 3. An aggregation of *Wulguru cuspidata* in a puddle at mid-tidal level near the mouth of Sandy Creek at Casuarina Beach, 26 July 2014. (Neil Wright) ◀

Figure 4. *Wulguru cuspidata* aggregating around the edge of a puddle at mid-tidal level near the mouth of Sandy Creek at Casuarina Beach, 7 December 2014. (Adam Bourke) ▲

Figure 5. *Wulguru cuspidata*, detail of a single individual shown in dorsal view, from Casuarina Beach, 7 December 2014. Note the nipple-like tail on the right. Scale bar = 1 mm. (Adam Bourke) ►

Figure 6. *Wulguru cuspidata*, detail of a single individual shown in dorsal view, from Casuarina Beach, 8 July 2014. Note the nipple-like tail on the right and some grains of quartz sand alongside. (Graham Brown) ►



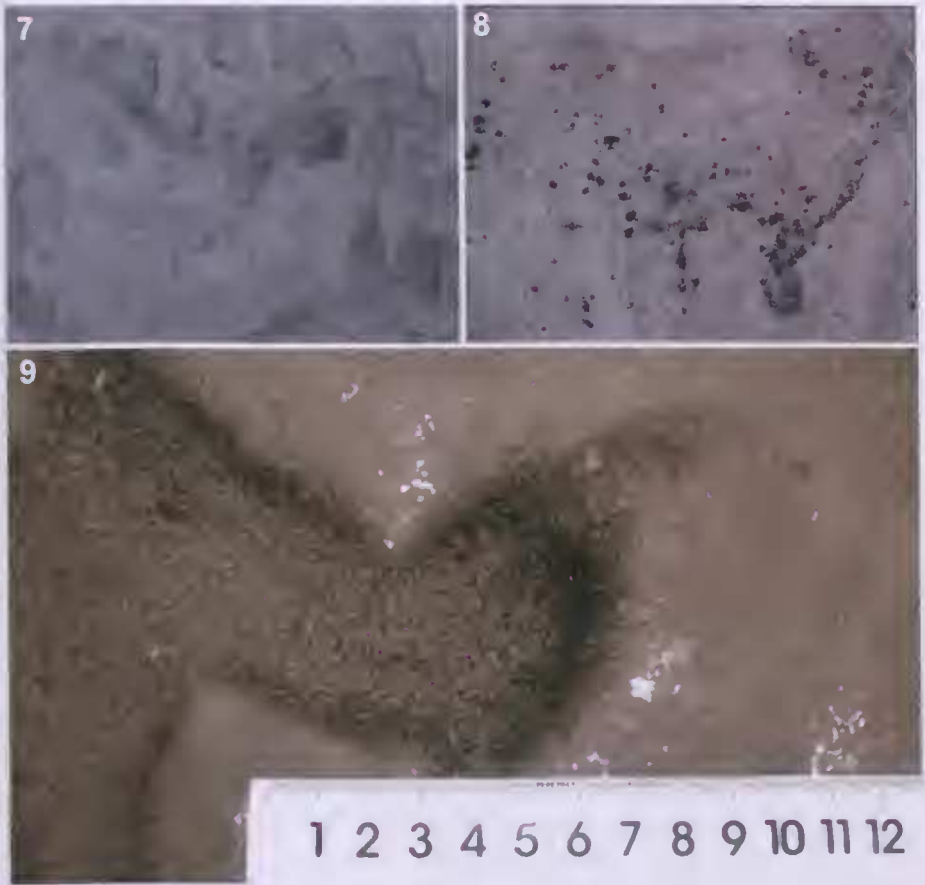


Figure 7. *Wulguru cuspidata*, near mouth of Sandy Creek, Casuarina Beach, 25 November 2014. These individuals have aggregated into a ring around the edge of wave-formed sand ripples at mid-tidal level. (Neil Wright)

Figure 8. *Wulguru cuspidata*, near mouth of Sandy Creek, Casuarina Beach, 7 December 2014. These individuals have aggregated into circular clusters on the floor of a wave-formed sand ripple at mid-tidal level following a heavy storm the previous night. (Neil Wright)

Figure 9. *Wulguru cuspidata*, near mouth of Sandy Creek, Casuarina Beach, 29 November 2014. Detail of individuals that have aggregated into a ring around the edge of a wave-formed sand ripple at mid-tidal level. (Neil Wright)

Following relaxation in magnesium chloride, some specimens from Casuarina Beach were preserved in 10% neutral buffered formalin and sent to Dr Leigh Winsor in Townsville for identification. He stained them with Gower's Carmine, cleared them in terpineol, and examined them microscopically. He identified them with confidence as *Wulguru cuspidata*, a species that he had named (Winsor 1988).

Besides recording the presence of *Wulguru cuspidata* for the first time in the Northern Territory, this article gives some general information on the morphology and biology of acoels as well as the family Convolutidae. Following that is a description of *Wulguru cuspidata* including our observations of its behaviour in the field. Finally we pose some of the questions resulting from these observations.

Identifying characters

Acoels (also known as acoelomorphs) are remarkable marine 'worms', morphologically, ecologically and phylogenetically. All of them are minute in size, usually being less than 5 mm long when adult, and the majority are barely recognisable by the human eye in the field. Though they have a mid-ventral mouth, acoels completely lack an intestinal cavity with the intestinal cells forming a solid mass containing many nuclei in the centre of the body. The gonads are not distinct from the surrounding body tissue (Barnes 1980). The body often ends in two pointed tails resembling those of a swallow. Those species that live in the intertidal zone, where the spaces between the sand grains are not continually filled with water, are the most specialised.

Though acoels are microscopic, their densities can be enormous (i.e. hundreds of thousands, perhaps millions, per square metre) (Figures 3, 4) and they are quite diverse numerically; currently there are about 400 named species. They will be overlooked in any standard survey of marine biodiversity because they are so small and because they pass through standard sieves, yet their enormous abundance means they are very important ecologically. The acoel fauna of Australia is poorly known. Presently there are seven species (in four genera) of free-living acoels known from Australia (Winsor 1989), and that paper contains a key to the genera in Australia.

Some convolutid acoels, including *Wulguru cuspidata*, harbour zoochlorellae (Volvocales) within their bodies (Figures 4–6), so are green in colour from the chlorophyll of these symbiotic microalgae. Juvenile convolutids are colourless when first hatched, but they quickly take on a green colour as their mesenchyme becomes filled with the microalgae they have acquired. After entering the convolutids, these microscopic algae lose their flagellae and undergo other changes but continue to carry on photosynthesis; that is, they become endosymbionts (Buchsbbaum 1964). They capture light, and the energy thus harnessed is passed to the tissues of the host organism. Therefore, convolutids can be described as 'solar-powered'. However, they can also feed autotrophically on diatoms in particular, or microscopic animals living in the water spaces between the sand grains (meiofauna).

An ecologically similar but more elongate green free-living acoel, though from a different family (Sagittiferidae), is the Mint Sauce Worm (*Symsagittifera roscoffensis*). This species is much better known under its former name of *Convoluta roscoffensis* and is a model organism for studying the development of bilateral animals. *Symsagittifera roscoffensis* is world-renowned for its behaviour. It occurs on sheltered sandy beaches with shallow water in the north-eastern Atlantic Ocean. When the tide recedes it gathers together at the surface of the puddles in hundreds of thousands to provide optimal photosynthetic conditions for its green symbiont *Tetraselmis convolutae* (Littlewood 2014).

Traditionally acoels were pigeonholed into the phylum Platyhelminthes (flatworms) as a group of Turbellaria (free-living flatworms) simply because they were small 'flat' soft-bodied metazoans. They were assumed to be turbellarians highly specialised through reduction in body size. However, molecular studies (Ruiz-Trillo *et al.* 1997) have shown they, and one other similarly anomalous group of 'flatworms', the Nemertodermatida, do not belong to the Platyhelminthes. Recent phylogenetic analyses based on these studies have proved equivocal with acoels either stemming from the base of the Bilateria or grouping with the Xenoturbellida, as sister group to the Ambulacraria (echinoderms and hemichordates) (Mwinyi *et al.* 2010).

The first species of free-living acoel to be described from Australia was *Heterochaerus australis* from Port Jackson, central New South Wales, in 1905 (Haswell 1905). The second, *Wulguru cuspidata*, was described on specimens collected from the intertidal zone of sandy beaches near Townsville in northern Queensland (Winsor 1988). The generic name *Wulguru* is derived from Wulguru Kaba, the name of a northern Queensland Aboriginal tribe. It pays respect to their sea country. The specific name is derived from the Latin word *cuspis*, meaning a point, and it alludes to the distinctive single pointed tail of this species.

Wulguru cuspidata is characterised by the following features (these are the ones that led Dr Winsor to identify our specimens with confidence): oblong shape with body terminating in a distinctive, pointed, nipple-like tail (Figures 4–6); lateral caudal lappets absent; body surface completely covered with cilia, with longer sensory cilia along the body margin; presence of symbiotic microalgae (of the genus *Platymonas*; hence its green colour); mid-ventral mouth (recognisable by the central brownish mass in Figure 6); single statocyst; three separate median ventral pores in the posterior third of the body – from front to rear – copulatory pore, then female pore, then male pore; muscular penis invaginated within a lightly muscular seminal vesicle; bursa copulatrix; two bursal canals.

Ecology

The central and western coast of the Northern Territory experiences a macrotidal regime with tides reaching a maximum range of 8.0 m, a mean spring tidal range of 5.5 m and a mean neap tidal range of 1.9 m (RAN Hydrographic Service 1973).

These extreme tides mean that the intertidal zone can be almost 2 km wide on springs, and is regularly 0.5 km wide on neaps. *Wulguru cuspidata* occupies only a narrow belt within that intertidal zone – barely a few metres wide – where the almost horizontal sand flats join the steeper-sloping high tidal ‘beach’ (Figure 1). When the tide recedes, temporary wave-formed ripples in the sand allow sea water to be retained at low tide as it percolates from the upper beach by gravity (Figures 1, 2). It is in these sand ripples parallel to the shore and in shallow water-filled runnels perpendicular to the shore, that *Wulguru cuspidata* lives. As mentioned above, individuals move continuously, but they cluster into a ring around the margin of these pools (Figures 7, 9) or, less frequently, into a circular mound (approx. 1 cm in diameter) on the floor of the puddles (Figure 8). The ring pattern can occur any time of the year, whereas the mounds only occur during the ‘build-up’ season, seemingly immediately after a heavy rainstorm.

Though densities fluctuate enormously, our surveys showed *Wulguru cuspidata* is present from July continuously through to January, and therefore they are probably present all throughout the year. Individuals tolerate high levels of sunlight, extremes of temperature, sudden bursts of heavy rain and longer periods of depressed salinity, low oxygen concentrations and shading brought about by the annual decomposition of *Sargassum* sp. seaweed in the dry season and the annual bloom of *Trichodesmium* cyanobacteria in the ‘build-up’. We observed that the major environmental factor affecting their occurrence and density was the reshaping of the beach brought about by the bouts of the north-westerly monsoon when the strong erosive waves and longshore currents associated with the monsoon completely reshaped the beach to a nearly flat profile by eliminating the slope at high tide and all the sand ripples and runnels at mid-tide and low tide. All the populations at Casuarina Beach vanished with the onset of the monsoon because that beach receives the full force of the monsoon and the reshaping was complete. However, the populations in Shoal Bay persisted because those beaches are sheltered from the full force of the monsoon and the puddles remained there.

The water in the puddles that *Wulguru* inhabit can become very hot on calm days. At midday on 25 October we measured the temperature at 33.78 °C (an average of five readings). However, these puddles were still cooler than those just 3 m seaward where they received no percolation of water from the beach. The temperature in these slightly larger pools was measured as 36.96 °C (an average of five readings) on the same occasion. A Mann-Whitney U Test yielded a P value of 0.009 (< 0.05), which led us to conclude the temperatures in the two sets of pools were significantly different to each other.

The individuals came to the surface about half an hour after the tide had receded beyond their puddles and they (at least we presume the same individuals) remained exposed there until it returned. They buried into the sand when the tide was in.

We checked this habit of burying by snorkelling at high tide, and there was not a single individual to be seen on top of the sand.

Gently touching these aggregations, or even disturbing the sand next to them, causes all the individuals to vanish beneath the sand at the point of impact and immediately around it. Presumably the worms sense the vibrations. However, they re-emerge quickly and reaggregate. Similarly, waving a hand just above the surface of the water in their puddles will cause most individuals to bury.

It would be interesting to observe a single puddle throughout the course of a low tide to see if the patterns formed by the constantly active *Wulguru* individuals remain constant or change.

There is one more side to the remarkable behaviour of these acoels. That is, individuals definitely aggregate at the surface when the tide is low at night just as they do during the day. We checked this by visiting Surf Lifesaver Creek at 8 pm on 9 August. The full moon had risen, and the density of individuals was the same as when the sun was fully out. Therefore, sunlight cannot be the only factor responsible for them emerging and aggregating during daylight.

Conclusions

Rather than this report closing one chapter on *Wulguru cuspidata* in tropical northern Australia by recording that it is present in great densities in the Darwin region, it actually opens the possibility for much research into the ecology and behaviour of these fascinating microscopic animals. There is a great deal more to be found out about them – their physiology, ecology and behaviour. How do they sustain their phenomenal density? Are they obliged to gain food solely from their algal symbionts or can they feed independently on even smaller animals living on the sand surface or deeper between the grains of sand? Why do they sometimes aggregate in rings and in dense circular patches at other times? How do they manage the environmental stressors of high temperatures, ultraviolet light, drying out, or the massively increased concentrations of toxins and nitrogen during the annual *Trichodesmium* bloom? Because they have a semi-permeable skin, would one expect them to take in diluted seawater by osmosis until they burst when there is a heavy shower when the tide is out? What biological clock is responsible for their daily and tidal behaviour? Is it linked to the cycle of the tides, but if so, it would seem counterproductive for them to use energy emerging on moonless nights, or indeed at night at all. What happens to them during the monsoon when their habitat is lost? And lastly, how do they recolonise the beaches when the monsoon is over?

What remarkable creatures they are!

Acknowledgements

We are greatly indebted to Leigh Winsor for identifying *Wulguru cuspidata* for us and also for reviewing the manuscript. Yen-Wei Chang undertook the statistical analysis on admittedly sparse data. Adam Bourke kindly took some magnificently detailed *in situ* photographs for us.

References

- Barnes R. D. (1980) *Invertebrate Zoology*. Fourth Edition. W.B. Saunders, Philadelphia.
- Buchsbaum R. (1964) *Animals Without Backbones: 1*. Penguin Books, Harmondsworth.
- Haswell W.A. (1905) Studies on the Turbellaria. *Quarterly Journal of Microscopical Science* 49, 425–467.
- Littlewood T. (2014) *Symsagittifera roscoffensis* (mint sauce worm). Nature-online. Natural History Museum, London. <<http://www.nhm.ac.uk/nature-online/species-of-the-day/biodiversity/climate-change/symsagittifera-roscoffensis/index.html>> (accessed 1 December 2014).
- Mwinyi A. *et al.* (2010) The phylogenetic position of Acoela as revealed by the complete mitochondrial genome of *Symsagittifera roscoffensis*. *BMC Evolutionary Biology* 10, 309.
- RAN (Royal Australian Navy Hydrographic Service) (1973) *Royal Australian Navy Hydrographic Service, Sheet AUS 27 (Approaches to port of Darwin)*. Commonwealth of Australia, Canberra.
- Ruiz-Trillo I. *et al.* (1997). Acoel flatworms: earliest extant bilaterian metazoans, not members of Platyhelminthes. *Science* 283, 1919–1923.
- Winsor L. (1988) A new acoel (Convolutidae) from the north Queensland coast, Australia. *Progress in Zoology* 36, 391–394.
- Winsor L. (1989) Marine Turbellaria (Acoela) from north Queensland. *Memoirs of the Queensland Museum* 28, 785–800.
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